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Short Communication

Phytochemical screening and isolation of a Prunoglycoside from *Mucuna pruriens (L.) DC* root

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ABSTRACT

Aim of the present study was to perform the extraction, isolation and phytochemical screening of methanolic extract of *Mucuna pruriens (L.) DC* roots. The methanolic extract was subjected to preliminary phytochemical screening. For the isolation of phytoconstituents, the extract was subjected to silica gel column chromatography. The isolated compound was characterized on the basis of spectral analysis. Fractionation of methanolic extract by column chromatography furnished a compound named Prunoglycoside. The structure of Prunoglycoside was elucidated as lanost-5-en-3β-ol-3-*O*-D-α-glucopyranoside.

1. INTRODUCTION

Mucuna pruriens (L.) DC commonly known as velvet seed, is the most popular drug in Ayurvedic system of medicine [1]. *Mucuna Pruriens (M. Pruriens)* is an annual, climbing shrub. The fuzzy hairs are available in young state but it is completely hairless during old age. The leaves are ovular in shape and leaflets are 2-3 mm long. The flower heads are take form of axially arrayed panicles with 15-32 mm long and have 2-3 or many flowers with lavender, white or purple colures. In the fruit ripening stage, a 4 to 13 cm long and 1 to 2 cm wide un-winged leguminous fruit develops [2]. All parts of *M. Pruriens* are generally used to treat impotence, diabetes mellitus, and several free radical mediated diseases such as rheumatoid arthritis, atherosclerosis, nervous disorders, analgesic, antipyretic activity and in the management of Parkinsonism [3-5]. The most important of the bioactive compounds of plants are alkaloids, lipids and phenolic compounds [6-8]. Seeds of velvet beans are known to produce the unusual nonprotein amino acid 3-(3,4- dihydroxyphenyl)-l-alanine (L-DOPA). It also contains glutathione, Gallic acid and beta-sitosterol. It has unidentified bases like mucunine, mucunadine, prurienine, prurieninine. Other bases isolated from the pods, seeds, leaves and roots include indole-3-alkylamines-N, N-dimethyltryptamine. Leaves also gave 6- methoxyharman. Serotonin is present only in pods. The seed also contains two tetrahydroquinoline alkaloid is namely (-) 3-methoxy-1, 1-dimethyl-6, 7-dihydroxy-1,2,3.4-tetrahydroquinoline and (-) 3-methoxy-1,1-dimethyl-7,8-dihydroxy-1,2,3.4-tetrahydroquinoline [9, 10].

2. EXPERIMENTAL

2.1 Chemicals and Reagents

The melting points were determined on a Perfit apparatus. The IR spectra were recorded in KBr pellet on Win IR FTS-135 instrument (Biorad, USA). Both ^{1}H and ^{13}C-NMR spectra were screened with a Bruker Avance 003 version NMR instrument operating at 400 and 100 MHz, respectively. The spectra were obtained in deuterated chloroform (DMSO-d_6) using tetramethyl silane (TMS) as internal standard, with chemical shifts (δ) expressed in ppm and coupling constants (J) in Hertz. ESI MS was scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA)

2.2 Plant material and preparation of extract

M. prurines roots were collected from the nearby region of H. N. B. Garhwal University Chouras campus, Srinagar, Garhwal, Uttrakhand, India in the month of July, 2010 and it was authenticated by Taxonomist, Department of Botany, H.

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N. B. Garhwal University, Uttarakhand. Voucher specimen was deposited of the same. The fresh roots were cleaned, dried in the sunlight and powdered through sieve #44 for uniform size. The ground root (2.5 kg) were extracted exhaustively first with hexane and then with methanol. The methanolic extract was concentrated under reduced pressure to yield (204 g, 8.1%) dark brown, viscous syrupy mass. The viscous dark brown mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. It was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol and their combinations in increasing order of polarity.

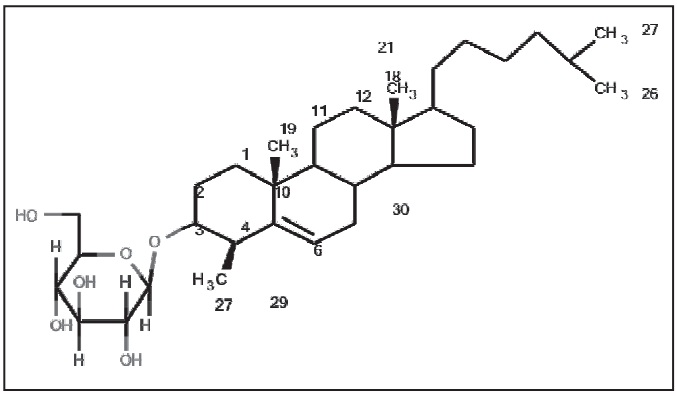

Fig. 1. Prunoglycoside **(**Lanosteryl glycoside**)**

3. RESULTS AND DISCUSSION

Prunoglycoside (Lanosteryl glycoside**): *MP:*** 258-260^0 C; ***UV*** λ_{max} ***(MeOH):*** 217nm **(**log e 4.3); ***IR*** ν_{max} ***(KBr):*** 3485, 3409,3350, 2930,1644, 1464, 1373, 1258, 1163,1071,1022, 1644 cm^{-1}; ***^{1}HNMR (DMSO-d_6):*** 5.24 (1H, brs, H-6), 4.73 (1H,d, *J*= 4.8 Hz, H-1'α), 4.67 ((1H, d, *J* = 4.8 Hz, H-2'), 4.18 (1H, dd, *J*=8.8,4 Hz, H-4'), 3.61 (1H, m, H-5') 3.44 1H,dd, *J*=4.8, 7.2 Hz, H-3β), 3.14 (1H,m, H-3'), 3.10 (1H, d, *J*=8.0Hz, H_2-6'a), 3.03 (1H, d, *J*=8.0 Hz, H_2-6'b), 1.16 (3H, brs, Me-19), 0.90 (3H, brs, Me-28), 0.84 (3H, d, *J*=6.4 Hz, Me-21), 0.77 (3H, d, J=7.2 Hz , Me-26), 0.75 (3H, d, *J*=7.2 Hz,Me-27), 0.71 (3H, brs, Me-29), 0.69 (3H, brs, Me-30), 0.59 (3H, brs, Me-18); ***^{1}HNMR (DMSO-d_6):*** Table 1; ***EIMS m/z (rel. int.):*** 590 [M]$^+$ (C_{36} H_{62} O_6) (3.7), 428 (48.9), 315 (100), 220 (3.7), 208 (3.9), 192 (4.8) ,180 (6.3), 152.

Lanosteryl glycoside (Fig. 1) was obtained as a colorless crystalline mass from chloroform: methanol (9:1) eluents. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3485, 3409. 3350 cm^{-1}) and unsaturation (1644 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 590 corresponding to the molecular formula of a triterpenic glycoside $C_{36}H_{62}O_6$. It indicated six double bond equivalents. Four of them were adjusted in a tetracyclic carbon framework of lanostane type triterpenic and the remaining one each in the olefinic linkage and glycoside moiety. Elimination of a glyosidic moiety yielded a prominent peak at m/z 428 related to lanostane. The important ion peak at m/z 100 [$C_{4,5}$-$C_{1,10}$ fission]$^+$ 152 [$C_{6,7}$-$C_{9,10}$ fission]$^+$, 192 [$C_{8,14}$-$C_{9,11}$ fission]$^+$ and 208, 220 [$C_{8,14}$-$C_{12,13}$ fission]$^+$ supported the existence of the vinylic linkage at C-5 and hydroxy group in the ring A, which was placed at C-3. The ^{1H} NMR spectrum of showed a one-proton broad signal at δ 5.24 assigned to vinylic H-6. A one- proton doublet at δ 4.73 (*J*=4.8 Hz) was ascribed anomeric 1'α –proton. Three one–proton doublets at δ 4.67 (J=4.8 Hz), 3.10 (*J*=8.0Hz) and 3.03 (*J*=8.0 Hz) were attributed to sugar proton H-2' and hydroxymethylene H_2-6' protons. A one-proton double doublets at δ 4.18 (*J*= 8.0, 8.4Hz) and two one-proton multiplets at δ 3.61 and 3.14 were accounted to hydroxymethine H-4', H-5', and H-3' protons, respectively. A one-proton double doublet a δ 3.44 with coupling interaction of 4.8 and 7.2 Hz was assigned to α-oriented H-3 carbinol proton. Three doublets at δ 0.84 (*J*=6.4 Hz), 0.77 (*J*=7.2 Hz) and 0.75 (*J*=7.2 Hz), all integrating for three protons, were attributed to secondary C-21, C-26 andC-27 methyl protons. Five three-proton signals at δ 1.16, 0.90. 0.71. 0.69 and 0.59 were associated with the C-19, C-28, C-29, C-30 and C-18 tertiary methyl protons, respectively. The remaining methylene and methine protons resonated between δ 2.97-0.93. The ^{13}CNMR spectrum of exhibited important signals for vinylic carbons at δ140.63 (C-5) and 121.64 (C-6), anomeric carbon at δ101.64 (C-1'), other sugar proton between δ 76.85 - 61.72, carbinol carbon at δ 77.73(C-3) and methyl carbons at δ 12.02 (C-18), 20.75 (C-19), 19.27 (C-21)19.49 (C-26), 12.13 (C-27), 28,64 (C-28), 27,08(C-29) and 18.94 (C-30). The ^{1}H and ^{13}C-NMR signals were compared with reported lanostane-type molecules. On the basis of spectral data analysis and chemical reaction, the structure of has been elucidated as lanost-5-en-3β-ol-3-*O*-D-α-glucopyranoside.

Table 1. ^{13}C- NMR values of Prunoglycoside **(**Lanosteryl glycoside**)**

C	δc	C	δc
1	36.67	19	20.75
2	34.09	20	31.79
3	77.73	21	19.27
4	42.28	22	40.72
5	140.63	23	23.19
6	121.64	24	36.31
7	24.33	25	29.71
8	43.51	26	19.49
9	50.05	27	12.13
10	38.81	28	28.64
11	21.67	29	27.08
12	29.06	30	18.94
13	45.65	1′	101.64
14	57.31	2′	76.85
15	31.84	3′	73.81
16	37.28	4′	70.52
17	50.71	5′	77.18
18	12.02	6′	61.72

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4. CONCLUSION

Phytochemical investigation of *M. pruriens* root showed the presence of alkaloids, glycoside, flavonoids, etc. Moreover the fractionation of methanolic extract by column chromatography furnished a compound named Prunoglycoside (Lanosteryl glycoside). The structure of Prunoglycoside has been elucidated as lanost-5-en-3β-ol-3-*O*-D-glucopyranoside.

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